

2<sup>nd</sup> try:

Yeasts Transformation and homologous recombination

Materials and Methods

➔ Yeast Protocol Handbook (Clontech)

We follow the same protocol as the first time but different DNA templates: we improved the PCR conditions and now have our fragments in higher concentrations and they seem quite pure.

Results

We obtained colonies on all plates corresponding to pbs2 +... constructs!

We inoculated them in selective medium and they grew again.

We still need to sequence the genomic DNA to be sure that they integrated the right sequences and that no mutation is present but we observed the pbs2GFP cells under the microscope and they do express the pbs2 gene fused to GFP:

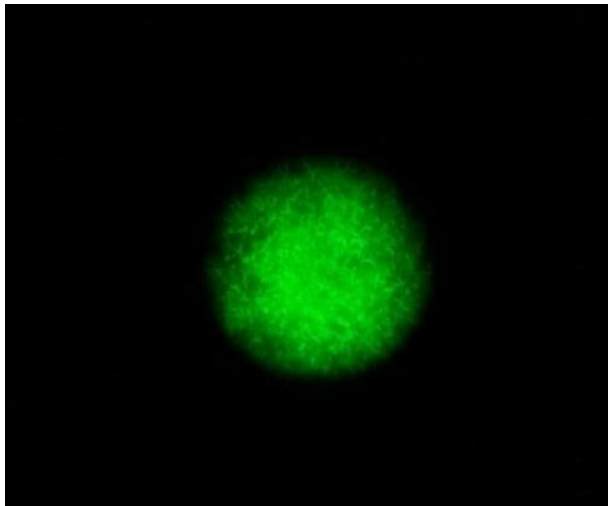


Figure 1 pbsGFPKan transformed yeasts 12.09.14

Strains we might have:

- Pbs2 GFP Kan
- Pbs2 Luc Kan
- Pbs2 splitGFP Kan
- Pbs2 splitLuc kan